Asian Journal of Biochemistry

ISSN 1815-9923

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Biochemistry 3 (2): 134-138, 2008
ISSN 1815-9923

Phytochemical Screening and Antibacterial Activity of *Tamarindus Indica* Pulp Extract

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Abstract: The phytochemical analysis and antibacterial activity of aqueous pulp extract of *Tamarindus indica* were studied. The aqueous pulp extract of this plant was obtained using hot water extraction method. The antibacterial activity of aqueous pulp extract of this plant was carried out against four bacteria; *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* by disc diffusion method. Phytochemical constituents present in the extract were found to include saponins (2.2%), alkaloids (4.32%) and glycosides (1.59%). Aqueous pulp extract of *Tamarindus indica* showed antibacterial activity against all the tested bacteria in the order of sensitivity as *Staphylococcus aureus*>*Escherichia coli*>*Pseudomonas aeruginosa* with the exception *salmonella typhi*. The antibacterial activity of aqueous pulp extract on *Staphylococcus aureus* was sensitive at 80, 120, 140, 160 and 180 mg mL^{-1} of extract with 0.2, 0.3, 0.6, 0.8 and 10.0 mm zones of inhibition while *Escherichia coli* revealed 0.2, 0.2, 0.4 and 0.6 mm zones of inhibition at 120, 140, 160 and 180 mg mL^{-1} of extract, respectively. *Pseudomonas aeruginosa* was only sensitive at 140, 160 and 180 mg mL^{-1} of the extract with 0.4, 0.6 and 0.8 mm zones of inhibition.

Key words: *Tamarindus indica*, pulp extract, rats, aqueous, zones of inhibition, antibacterial activity

INTRODUCTION

Traditionally, the use of plant preparation as sources of drugs are based on the experience and superstition passed from generation to generation, virtually by the word of mouth (Sofowora, 1993). Plants have provided a source of inspiration of novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is two fold in the development of new drugs: (1) they may become base for the development of a medicine, i.e., natural blue print of the development of new drugs or (2) a phytomedicine to be used for the treatment of disease.

Tamarindus indica (Linn.), family Fabaceae/Leguminosea (Caesalpiniodae), is a slow growing, long lived massive fruit tree of the tropics. Although native to tropical Africa, the tree grows wild through out Sudan and was so long ago introduced into and adopted in India that it has often been reported as indigenous there also. In all tropical and near tropical areas, it is grown as a shade and fruit tree along roadsides, indoor yards and parks.

Medicinal uses of *Tamarindus indica* are numerous. The fruit extracts are used as refrigerants in fevers and as laxatives and carminatives alone or combinations with limejuice, honey, milk, dates spices and camphor. The pulp is used in digestive, as remedy for biliousness and bile disorders (Jayaweera, 1981). As an anti scorbutic, it is applied to heal inflammations and soar throat, mixed with salt to treat rheumatism and administered to alleviate sunstroke, dasine poisoning and alcoholic intoxication in Southeast Asia (Morton, 1987).

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The pulp is composed of tartaric acid, citric and malic acids, potassium bitartarate, pectin, gum, water and parenchymatous fiber (Nyadoi and Abdullahi, 2004). It is used to allay thirst, is nutritive and forms useful drinks given to persons recovering from sickness to keep their bowels regular (Morton, 1987). Punch made from it in mixture with other beverages is used to allay scalding urine in West Indies (Morton, 1987). In Mauritius the Creoles mix the pulp with salt to rheumatism while a decoction from the bark is used in asthma treatment. The Bengalese uses the pulp in dysentery treatment and as food (pods boiled or macerated) in food scarce periods.

In Nigeria, particularly in the northwestern part, *Tamarindus indica* pulp is popularly employed on a daily basis as a flavor in the production of local drinks, preservation of food and in the general traditional medicine practice as a drug conveyor, in combination with other herb for treatment of various diseases such as indigestion, constipation, fever and inflammation. In the native practice, *Tamarindus indica* is applied on inflammations, used as a gargle for sore throat and mixed with salt as a liniment for rheumatism, accelerate expulsion, relieve pains, reduce secondary bacterial infections and promote healing (Fabiyi *et al*., 1993).

Because of the side effect, resistance and pathogenic microorganism build against the antibiotics, much recent attention has been paid to extracts of biologically active component isolated from plant species used in herbal medicine. Medicinal plants may offer a new source of antibacterial, antifungi and antiviral activities.

In the present study therefore, *Tamarindus indica* pulp extract, were screened for phytochemical constituents and antibacterial activity.

MATERIALS AND METHODS

Plant Material

Tamarindus indica pulp was obtained from the wild of Sokoto south local government area of Sokoto state, Nigeria. The plant was identified at Botany unit, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen was prepared and deposited in the herbarium of the same institution for reference as recommended by Kumar *et al*. (2000).

Preparation of Extract

The aqueous extract of *Tamarindus indica* was obtained using the hot water extraction technique in order to stimulate the local procedure as described by Akinyole and Olorede (2000). Four hundred 400 g soaked in 2 L of distilled water and boiled for 5 min. This was shaken for 10 min and allowed to cool then filtered. The filtrate was evaporated to a residue in a drying cabinet. The percentage yield was 25.2%w/w.

Phytochemical Screening

The possible presence of saponins and glycosides in the pulp were screened for according to the procedure as described by Trease and Evans (1986), while the presence of alkaloids was assayed using the method described in Sofowora (1993). This was carried out in Biochemistry Department of Usmanu Danfodiyo University, Sokoto, Nigeria in May 2006.

Microorganisms Tested

The following bacterial cultures were used: *Escherichia coli*, *Staphlococcus aureus*, *Pseudomonas aeruginosa* and *Samonella typhi* were obtained from Microbiology Department, Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto and reidentified according to the method of Cowas and Steel (1992).

Antibacterial Testing

Antibacterial activity of the aqueous extract was determined by the disc diffusion method as described by Lennette (1985). The microorganisms were cultured overnight at 35°C in nutrient agar. Suspension of the bacteria with an optical density of McFarlend 0.5 was made in isotonic sodium chloride solution.

Petri dishes with 60 mL of sterile Mueller Hinton agar were seeded with the appropriate bacterial suspension. Sterile, 6 mm diameter filter paper disc were impregnated with the extract of different concentration, gently tapped to remove excess liquid and positioned on seeded plates at 45° opposite each other into each petri dish, respectively. After incubation for 24 h at 35°C, the plates were observed for zones of inhibition and the diameter of these zones measured in millimeters.

This research was conducted in the Microbiology unit of Biological Science Department of Usmanu Danfodiyo University, Sokoto, Nigeria in May 2006.

RESULTS

The result of the phytochemical screening of *Tamarindus indica* pulp extract qualitatively and quantitatively is shown in Table 1 and 2, respectively. The phytochemical constituents of the extract included alkaloid (4.32%), saponins (2.2%) and glycosides (1.59%). Tannins and flavoniod were not detectable at the tested assay condition.

Determination of the inhibition zones by means of the disc diffusion method (Table 2) shows that the pulp extract exhibited an antibacterial effect against all the four tested bacterial except *Samonella typhi*. *Staphylococcus aureus* was sensitive at 80,120, 140, 160 and 180 mg mL^{-1} of extract with 0.2, 0.3, 0.6, 0.8 and 10.0 mm zones of inhibition while *Escherichia coli* revealed 0.2, 0.2, 0.4 and 0.6 mm zones of inhibition at 120,140, 160 and 180 mg mL^{-1} of extract respectively. *Pseudomonas aeruginosa* was only sensitive at 140, 160 and 180 mg mL^{-1} of the extract with 0.4, 0.6 and 0.8 mm zones of inhibition.

Table 1: Phytochemical screening of *Tamarindus indica* pulp

Phytochemical groups	Results
Alkaloid	+++
Anthraquinone	+++
Tannin	-
Saponin	+++
Flavonoid	-
Glycoside	+++
Cardiac glycoside	+
Flavonoid glycoside	-
Anthraquinone glycoside	+
Saponins glycoside	+
pH	1.27

+++: Represents high concentration; +: Represents low concentration; -: Represents constituents not detectable using the specified assay method

Table 2: Antibacterial activity of *Tamarindus indica* pulp extract

	Zone of inhibition (mm)				
Test bacteria	80	120	140	160	180
Concentration of extract in mg mL^{-1}					
E. coli	-	0.2	0.2	0.4	0.6
S. aureus	0.2	0.3	0.6	0.8	1.0
P. aeruginosa	-	-	0.4	0.6	0.8
S. typhi	-	-	-	-	-

DISCUSSIONS

In literature it has been indicated that medicinal plants are the backbone of traditional medicine (Fransworth, 1994) and the antibacterial activity of plant extract is due to different chemical agent in the extract, which were classified as active antimicrobial compounds (Rojas *et al*., 1992). Plants have the capacity to synthesize a diverse array of chemicals and understanding how phytochemicals function in plants may further our understanding of the mechanisms by which they benefit humans. In plants, these compounds function to attract beneficial and repel harmful organisms, serve as photoprotectants and respond to environmental changes. In humans, they can have complementary and overlapping actions, including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, reduction of inflammation, modulation of steroid metabolism and antibacterial and antiviral effects (Johanna, 2003).

In the present study, aqueous pulp extract of *T. indica* tested positive for the presence of alkaloids (4.32%), saponins (2.2%) and glycosides (1.59%). Alkaloid is a plant-derived compound that is toxic or physiologically active, contains nitrogen in a heterocyclic ring with complex structure and is of limited distribution in the plant kingdom. Alkaloids are formed as metabolic by-products and have been reported to be responsible for the antibacterial activity (Doughari, 2006). This is consistent with the reports of De *et al*. (1999) of which 35 different Indian spices and herbs indicated that *Tamarindus indica* pulp among others, had potent antimicrobial activities against the test organisms *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae*. Many phytochemicals are present in plants as glycosides (with a sugar moiety attached). Generally, glycosides are nonvolatile and lack fragrance. Cleaving the glycosidic bond yields the aglycon, which itself may be volatile and fragrant. Glycosides serve as defense mechanisms against predation by many microorganisms, insects and herbivores (De *et al*., 1999). This may therefore explain the demonstration of antimicrobial activity by the stem bark and leaf extracts of *Tamarindus indica* (Doughari, 2006).

The low percentage yield of 25.2% suggest that the plant *Tamarindus indica* contains fibers which will turn out to be residue after extraction while the average pH is 1.27 indicating the acidic nature of the extract. This acidity could be as a result of the presence of hetero-organic acids in *Tamarindus indica* pulp as reported by Morton (1987). The acidic nature and the antibacterial activity of *Tamarindus indica* obtained from the result suggests its usefulness in preservation of food items against contamination by *E. coli* and other gram negative rods as well as in the management of cases of food infection by this pathogens (Isu, 2005).

The aqueous pulp extract of *Tamarindus indica* exhibited increasing degree of antibacterial activities against the tested microorganisms in the following order of sensitivity; *Staphylococcus aureus* >*Escherichia coli*>*Pseudomonas aeruginosa*. The sensitivity of *E. coli* to *Tamarindus indica* is consistent with the observation that *E. coli* and a few other gram negative bacteria are affected by some plant extract (Chlabra *et al*., 1981). This inhibitory effect may be as a result of the change in pH and chemical constituent(s) of the aqueous pulp extract of this plant. The mechanism of action of these phytochemicals may be via lysing the cell, increasing permeability of the cell wall and membrane, inhibition of protein and DNA synthesis and or by inhibiting the transport of nutrient across the cell wall or membrane (Stewart and Beswick, 1979). This inhibitory effect of the extract on the growth of these microorganisms could be attributed to the presence of some phytochemicals that were found present in the plant extract. Saponins has detergent properties and serve as lytic agent and exhibits anti inflammatory properties (Lewis and Elvin-Lewis, 1977) while alkaloids and glycosides among functions with the aid of their defense mechanism act as phytoprotective agent against invading microorganism. The demonstration of antibacterial activity against both gram positive and gram negative bacteria by this plant may be indicative of the presence of broad spectrum antibiotic compounds (Doughari, 2006).

The success of the ethnobotanical approach to drugs discovery can no longer be questioned. Historical and current discoveries demonstrate its power (Cox, 1994). A complete study conducted

with the purpose of finding these chemicals is worthwhile. The optimal effectiveness of a medicinal plant may not be due to one main active constituent, but may be due to the combine action of different compounds originally in the plant (Bai, 1990).

From this study we can conclude that this is a promising plant and the result confirms the use of this plant in traditional medicine for the treatment of infections.

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